Sandra Magina, Ana P. Marques and Dmitry V. Evtuguin*

Study on the residual lignin in *Eucalyptus globulus* sulphite pulp

Abstract: The residual lignin (L_R sample) was isolated from unbleached acid sulphite pulp from *Eucalyptus globulus* with kappa number 18.2 by acidolysis and structurally characterized by wet chemistry and NMR techniques. The main structural features of L_R were compared with lignin isolated from sulphite spent liquor (L_{SSL} sample) and dioxane lignin (DL sample) from eucalypt wood. L_R contains less sulphonic groups (4.4%) compared to L_{SSL} (11.3%), and its molecular weight (2200 Da) is very close to that of DL (2600 Da). A part of sulphonic groups is located at the benzylic carbon in *β-O-4′* and *β-5′* structures. L_R revealed ca. 20% lower abundance of *β-O-4′* structures than DL, but ca. 40% higher abundance of these structures than L_{SSL}. The degree of condensation of L_R was higher than that of DL but lower than that of L_{SSL}. The condensed structures in L_R mainly originate from C6-linked syringyl units. The structural peculiarities of L_R consisted of the relatively high proportion of syringyl units compared to DL and the strong structural association with hemicelluloses via benzyl ether linkages. The differences in the structure of residual lignins from eucalypt sulphite and kraft pulps have been discussed.

Keywords: acid sulphite pulping, *Eucalyptus globulus*, lignosulphonate, NMR, residual lignin

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Introduction

Acidic sulphite pulping of *Eucalyptus globulus* allows sustainable production of total chlorine free (TCF) bleached pulps (both paper grade and dissolving pulps). The annual production of bleached sulphite eucalypt pulps exceeds 1.3 Mt year^{-1} in South Africa, Portugal, Spain and Brazil. The general understanding and further improvement of pulping/bleaching performance needs detailed information on both the lignin reactions occurring during pulping and the structure of residual lignin (RL) in pulps. Despite numerous studies on the chemistry of sulphite delignification (Glennie 1971; Gierer 1982; Sjöström 1993), the basic information on the structure of dissolved lignins in spent sulphite liquors (SSL) and, especially, of RL in pulps still needs to be clarified. Historically, lignosulphonates (LS) from sulphite pulping of softwoods (mainly spruce) were considered for structural studies and applied research, whereas this knowledge for the hardwood pulping is rather limited (Sjöström 1993). A study on LS from the magnesium-based acid sulphite pulping of *E. globulus* revealed that lignin degradation pathways are different from those reported for softwoods (Marques et al. 2009a,b).

There is little information available regarding the structure of RL in sulphite chemical pulps (Kallmes 1960; Wood and Goring, 1973; Koch et al. 2003). It was suggested, however, that its sulphonation degree is relatively low and that its molecular weight and condensation degree are higher than those in wood lignin (Rydholm 1965; Glennie 1971; Berry and Bolker 1987). The proportion of different structural units in sulphite pulp might be different from that in wood (Glennie 1971). For example, the ratio between syringyl (S) and guaiacy (G) units (S/G ratio) in sulphite poplar (*Populus* spp.) pulp is higher than that in wood lignin (Stone 1955). Probably, the delignification of vessels, rays and middle lamellae, which are rich in G units, is easier than that of the secondary wall of libriform fibres enriched in S structural units (Glennie 1971).

Sulphite pulps are much easier to bleach in short TCF sequences (e.g., Eop-P, O-Q-P, Paa-Q-P, Z-Eop-Po, etc.) than kraft ones (Fletcher et al. 1997; Süss 2006; Germer and Hostachy 2011). This is usually explained by the higher brightness of sulphite pulps compared to kraft pulps and by the different origin of RL. It is generally assumed that RL in sulphite pulps should be less condensed and partially sulphonated, and therefore it can be more easily removed in alkaline bleaching stages than the RL of kraft pulps (Süss 2006). However, to date, there is no experimental evidence on the structural features and molecular weights in this context. Meanwhile, this information is extremely important for the development of new

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bleaching strategies to produce, for example, dissolving sulphite bleached pulps, for which lower levels of RL are tolerable than for normal paper grade pulps.

The main goals of this work were to evaluate the structural features of RL in sulphite *E. globulus* pulp and to infer the changes occurred in lignin during acidic sulphite pulping. The known structure of eucalypt wood lignin was compared with the structures of LS dissolved in SSL and with the RL in pulp. Lignins were isolated from pulp by soft acidolysis and from SSL by dialysis followed by freeze drying. The main structural features were evaluated by wet chemistry methods and 1D and 2D NMR spectroscopy, and the molecular weight were assessed by size exclusion chromatography (SEC).

Materials and methods

The experimental outline is illustrated in Figure 1. Industrial thin spent liquor (SSL) and unbleached washed sulphite pulp with 18.2 kappa number (κ) from magnesium-based acidic sulphite pulping of *E. globulus* were supplied by Caima Cellulose Company S.A. (Constância, Portugal). Pulp was additionally washed with distilled water (1:100) and air-dried. Thin SSL was purified to obtain the lignosulphonate-rich fraction (L_{SSL} sample) by dialysis with a membrane of 5000 nominal molecular weight cut-off (NMWCO) (Pierron) against distilled water for 8 h followed by freeze-drying of dialysate (Marques et al. 2009b). The RL from pulp (L_R sample) was isolated by soft acidolytic treatment (Evtuguin et al. 2001), which was adopted from Pinto et al. (2003a,b). Typically, 22.0 g of air-dried extractives-free (Soxhlet-extracted by acetone, 4 h) sulphite pulp was submitted to three sequential extractions (each of 45 min) under reflux with 500 ml of 9:1 dioxane:water solution (v/v) containing 0 1 M of HCl, under N_2 atmosphere. The resulting extracts were further concentrated separately to a total volume of ca. 150 ml. Finally, lignin was precipitated in distilled cold water (1.5 L), centrifuged and washed until neutral pH of filtrates with distilled cold water and vacuum-dried at room temperature (ca. 20°C). The yield of L_R was ca. 300 mg.

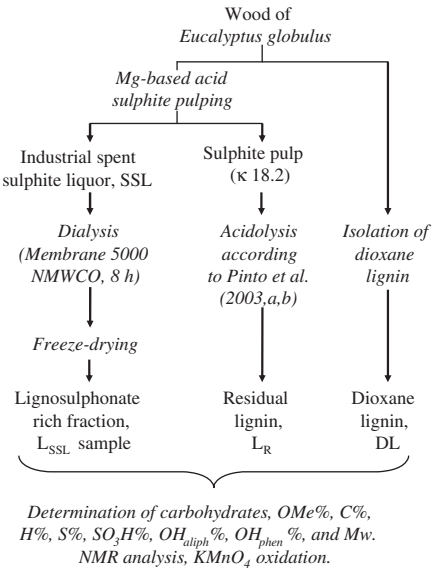


Figure 1 Outline of the experimental procedure.

L_{SSL} and L_R were analysed for ash (calcinations at 525°C according to Tappi T 211 om-93) and residual sugars as alditol acetates by GC (Evtuguin et al. 2003). The elementary analysis was performed on a CHNS-932 elemental analyser (LECO Corp., St. Joseph, USA). The functional analysis for sulphonic and phenolic groups was carried out by potentiometric titration, and the methoxy groups were determined by the Zeisel-Vieböck-Schwappach method (Zakis 1994). The permanganate oxidation (PO) of isolated L_{SSL} and L_R was performed as described previously (Gellerstedt 1992). The oxidation products of preliminary ethylated lignins were identified by GC (Trace Gas Chromatograph 2000 series, Thermo Scientific, Austin, USA) equipped with a mass spectrometer (Thermo Scientific DSQII, Austin, USA). The chromatographic conditions were 180°C → 290°C (4°C min^{-1}); injector temperature 250°C. L_R was acetylated by Ac_2O with pyridine as catalyst (Zakis 1994).

The quantitative ^{13}C NMR spectra and qualitative ^1H NMR spectra of L_{SSL} in D_2O (323 K) and acetylated L_R in dimethyl sulphoxide-d_6 (DMSO-d_6) (318 K) were recorded on a AVANCE 300 spectrometer (Bruker, Wissembourg, France) operating at 300 13 MHz for proton and 75.47 MHz for carbon in acetone (for L_{SSL}) or TMS (for L_R) as internal references in 5-mm-diameter tubes. The concentrations of L_{SSL} in D_2O were 2.5% for proton and ca. 30% for carbon spectra and concentrations of L_R in DMSO-d_6 were 2.0% for proton and ca. 25% for carbon spectra. The conditions for the proton spectra were as follows: relaxation delay of 2.0 s and about 200 scans were collected (60° pulse). The quantitative carbon NMR spectra were acquired with a pulse width of 4.8 μs (90° pulse), a relaxation delay of 16 s and 18,000 scans collected.

2D ^1H NMR (absolute-mode COSY) and the phase-sensitive ^1H-detected heteronuclear single quantum coherence (HSQC) spectra were recorded on a BRUKER AVANCE 300 spectrometer at 323 K. COSY spectra were recorded acquiring 2K×512 increments transformed to a 2K×1K data matrix after zero-filling, FT and squared sine-bell apodization applied to both dimensions. COSY spectra were acquired over a 9.0 ppm window in both F2 and F1 directions. For each t_1 value 600 scans were accumulated. The HSQC spectra were acquired over an F1 spectral weight of 12,000 Hz and an F2 width of 2000 Hz with a 2048×1024 matrix and 128 transients per increment. The delay between scans was 2 s and the delay for polarization transfer was optimized for $^1J_{C\,H}$=148 Hz.

Details of SEC of L_{SSL}: A PL-GPC 110 system (Polymer Laboratories, Shropshire, UK) was available equipped with two PL aquagel-OH MIXED 8 μm, 300 mm×7.5 mm columns protected by a PL aquagel-OH Guard 8 μm pre-column. The columns, injector system and the detector (RI) were maintained at 36°C during the analysis. The eluent (0.1 M aqueous solution of $NaNO_3$) was pumped at a flow rate of 0.9 ml min^{-1}. The analytical columns were calibrated with PSSNa standards (Pressure Chem. Comp., Pittsburgh, USA) in the range of 1–100 kDa. L_R was analysed in the same instrument equipped with two PLgel 5 μm MIXED-D 300 mm×7.5 mm columns protected by a PLgel 5 μm pre-column (Polymer Laboratories Ltd, Shropshire, UK) at 70°C. The eluent (0 1 M LiCl in DMF) was pumped at a flow rate of 0 9 ml min^{-1}. The column was calibrated with lignin model compounds (monomers, dimers and tetramers) and lignin samples (Mp=950–3200 Da) previously characterized by ESI-MS (Evtuguin et al. 1999).

Results and discussion

Molecular weight of residual lignin (L_R)

Lignin from unbleached sulphite eucalypt pulp of κ 18.2 was isolated by a modified acidolytic technique previously described for kraft pulps (Pinto et al. 2003a). The molecular weight (Mw) of residual lignin (L_R sample) was lower than that reported for dioxane lignin (DL sample) isolated from eucalypt wood (*E. globulus*) by the same technique (Table 1). This fact may be due to partial degradation of lignin remained in pulp during acidic sulphite pulping. The determination of Mw of lignin dissolved in sulphite spent liquor (L_{SSL} sample) is difficult without its previous purification. Due to the presence of significant amount of sugars, inorganic salts and lignin degradation products with low molecular weight in SSL, the Mw of LS was unreasonably low (ca. 950 Da). Accordingly, LS was purified by dialysis with a membrane of 5000 NMWCO. About 40% of SSL dry matter was removed during SSL dialysis. Marques et al. (2009b) found that besides sugars and inorganics, the highly sulphonated monomeric/dimeric structures are removed during dialysis with this membrane and retentate becomes enriched in oligomeric LS. The Mw of purified LS was 2350 Da, which is almost triple of that of purified lignin isolated from kraft black liquor (Pinto et al. 2003a). It was confirmed that L_{SSL} possesses significantly higher molecular weight compared to kraft lignin. However, the Mw of L_R in unbleached sulphite is very similar to that in kraft pulps (κ 14–18) (Pinto et al. 2002b) (2200 and 2170 Da, respectively).

Table 1 Results on the chemical analysis of L_{SSL} and L_R.

Indices	L_{SSL}	L_R	DL[a]
Ash (%)	6.9	2.9	0.4
Carbohydrates (%)	4.1	6.2	0.9
Rhamnose	0.3	0.0	0.0
Xylose	2.7	4.0	0.5
Mannose	0.2	0.1	0.0
Galactose	0.5	0.1	0.1
Glucose	0.4	2.0	0.3
C (%)	48.2	54.9	60.8
H (%)	6.6	6.1	6.4
S (%)	5.5	1.8	–
SO_3H (%)	11.2	4.4	–
Phenolic OH (%)	1.4	2.2	1.9
OCH_3 (%)[b]	18.4	20.7	23.8
M_w (Da)	2350	2200	2600

[a]Evtuguin et al. (2001).

[b]Corrected for the ash and sugar contents.

Wet chemistry analyses

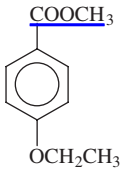
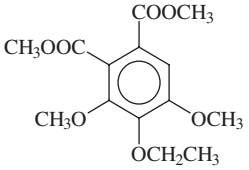
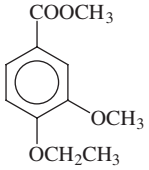
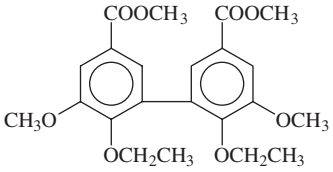
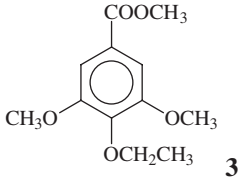
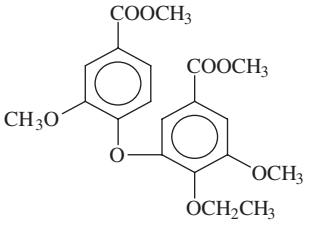
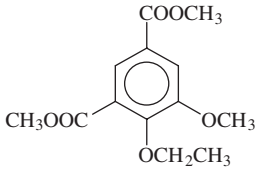
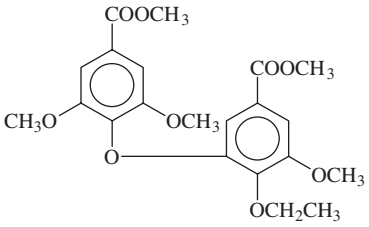
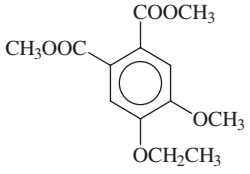
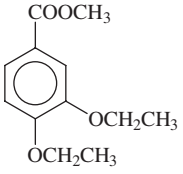
The results of chemical analyses of L_{SSL} and L_R are presented in Table 1. These are compared with previously reported data of DL isolated from *E. globulus* wood (Evtuguin et al. 2001). It may be concluded that L_{SSL} and L_R have less OMe groups compared to DL and are partially sulphonated. The amounts of sulphonic groups, however, were almost triple in L_{SSL} compared to that in L_R, which was water-insoluble and incompletely soluble in DMSO. L_R contains almost 50% more phenolic groups than L_{SSL}, indicating a more ramified structure of the former.

The information about different "condensed" and "non-condensed" structures in L_{SSL} and L_R was assessed based on degradation products arising from $KMnO_4$ oxidation (PO) (Table 2). Lignins were ethylated prior to oxidation to protect phenolic units against aromatic ring degradation and to be able to distinguish PO products with free phenolic groups. Nearly 96% of PO products were identified. Products **1, 2** and **3** were assigned to non-condensed lignin structures derived from the *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) structural units, respectively. The isohemipinic acid methyl ester (**4**) is originated from degraded phenylcoumaran (*β*-5′) structures and the products **5** and **6** are assigned to G and S units, respectively, condensed at the C-6 position. Other condensed structures of biphenyl and diaryl ether types are presented by products **7** and **8**/**9**, respectively (Table 2). The product **10** may be assigned both to demethoxylated G units and to condensed tannins of catechin type (Marques et al. 2009a).

The comparison of PO degradation products **1**–**10** from L_R and L_{SSL} is very conclusive. L_R contains less amounts of phenolic non-condensed G units (product **2**) compared to L_{SSL} and DL, and higher amounts of S units (product **3**). The higher abundance of S structures in L_R than in L_{SSL} is obvious. At the same time, unlike RL in kraft pulp (Pinto et al. 2003a), no increase of biphenyl and diaryl ether structures has been detected in L_R in comparison to DL isolated from wood. However, L_R demonstrates an impressive increase of S-type structures condensed at C-6 of an aromatic ring; more precisely, the abundance of structures **6** was almost double those in wood and almost 50% higher than in L_{SSL}. Hence, syringyl structures in L_R of sulphite pulp are highly condensed.

Unlike DL and L_{SSL}, L_R contained rather significant amounts of carbohydrates (mainly xylan and glucan) that could not be removed upon purification and which are probably chemically linked to lignin (Table 2). A similar observation was made previously based on NMR studies of eucalypt LS isolated from SSL (Marques et al. 2009b).

Table 2 Results on permanganate oxidation analysis of L_{SSL}, L_R and DL.

Oxidation product	(mol%)	Oxidation product	(mol%)
 **1**	L_{SSL}: 2 L_R: 1 DL: 3	 **6**	L_{SSL}: 8 L_R: 11 DL: 6
 **2**	L_{SSL}: 24 L_R: 14 DL: 18	 **7**	L_{SSL}: <1 L_R: <1 DL: 2
 **3**	L_{SSL}: 54 L_R: 67 DL: 58	 **8**	L_{SSL}: <1 L_R: <1 DL: 1
 **4**	L_{SSL}: 3 L_R: 1 DL: 3	 **9**	L_{SSL}: 2 L_R: 4 DL: 7
 **5**	L_{SSL}: 5 L_R: 3 DL: 3	 **10**	L_{SSL}: 2 L_R: 1 DL: 0

Apparently, strong association with carbohydrates, condensation reactions and limited lignin sulphonation are the main reasons for the retention of LR in pulp.

Analyses by NMR

The main structural features of L_{SSL} and L_R were evaluated by quantitative ^{13}C NMR (Figures 2 and 3). L_{SSL} was dissolved in D_2O and acetylated L_R in DMSO-d_6. Acetylation was necessary to improve solubility in DMSO, because quantitative ^{13}C NMR spectroscopy needs high lignin concentration in solution (ca. 30%). The assignments of signals in ^{13}C NMR spectra are based on the analysis of proton-carbon correlation (HSQC) 2D NMR spectra of corresponding lignins (Figures 4 and 5). Moreover, the published database of sulphonated eucalypt lignins (Luthaes et al. 2008; Marques et al. 2009b) and native eucalypt lignins (Evtuguin et al. 2001) served for interpretation. The assignments were additionally confirmed by the single-bond proton-proton correlation spectra (COSY, spectra are not shown) and HSQC spectrum of non-acetylated L_R (Figure 6). The signal assignments of acetylated L_R relies on databases published for model compounds (Ralph et al. 2004) and hardwood/softwood lignins (Kilpeläeinen et al. 1994; Ämmälahti et al. 1998). The signals belonging

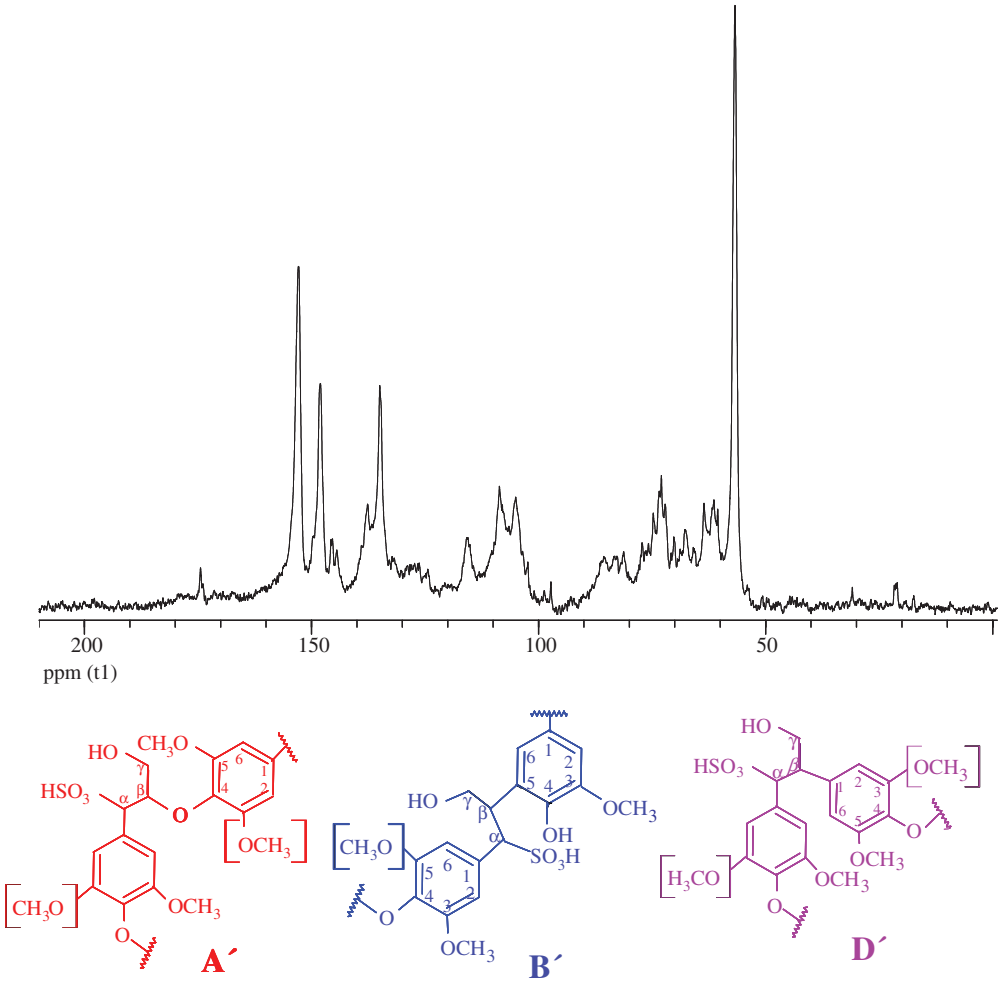


Figure 2 Quantitative ^{13}C NMR spectrum of L_{SSL} (D_2O, 50°C). Structures **A′**, **B′** and **D′** designate sulphonated *β-O-4′*, *β-5′* and *β-1′* structures, respectively.

to concomitant carbohydrates were assigned according to known data on non-acetylated and acetylated xylan (Evtuguin et al. 2003; Miyagawa et al. 2013) and glucan (Bock et al. 1984; Lisboa et al. 2005).

The analysis of the main lignin structures in L_{SSL} and L_R is summarized in Table 3. The calculations have been carried out per one aromatic ring according to previously established methodology (Evtuguin et al. 2001; Marques et al. 2009b). The aromatic region equivalent to six carbon atoms was integrated at 103–156 ppm. The abundance of lignin structures linked by *β-O-4′* bonds was calculated based on signals integrals at 61.0–63.0 ppm in acetylated L_R or at 60.0–62.0 ppm in L_{SSL} (Cγ resonance in *β-O-4′* structures). Similarly, the abundance of phenyl coumaran (*β-5′*) and pino/syringaresinol (*β-β′*) structures was assessed based on Cβ resonances in corresponding structures (Figures 4 and 5).

The abundance of *β-O-4′* structures in L_{SSL} was much lower than that in L_R, which contained in turn about 20% less amount of these structures than wood lignin (Table 3). Interestingly, the relative occurrence of *β-O-4′* structures found in L_{SSL} and L_R was comparable to those detected previously in purified lignin from kraft black liquor and in RL of kraft pulp with κ ca. 14 (Pinto et al. 2003b). This means that *β-O-4′* bonds were cleaved to a similar extent in kraft and acidic sulphite cooking of eucalypt wood. The principal difference between *β-O-4′* structures of L_{SSL} and L_R consisted in the amount of sulphonic groups at the benzylic carbon (BnC). According to rough estimation made by the volume of Cβ peaks in the HSQC spectra of lignins (Figures 4 and 5), more than 95% of *β-O-4′* structures in L_{SSL} are sulphonated at the Bn position (structures **A′**, Figure 2), whereas about 90% of *β-O-4′* structures in L_R were not sulphonated (structures **A**, Figure 3). Other structures of L_R containing sulphonic groups are *β-5′* units that are almost completely sulphonated at the Bn position. This conclusion could be drawn based on the absence of the cross-signal of Cα in corresponding **B** structures (Figure 3) of

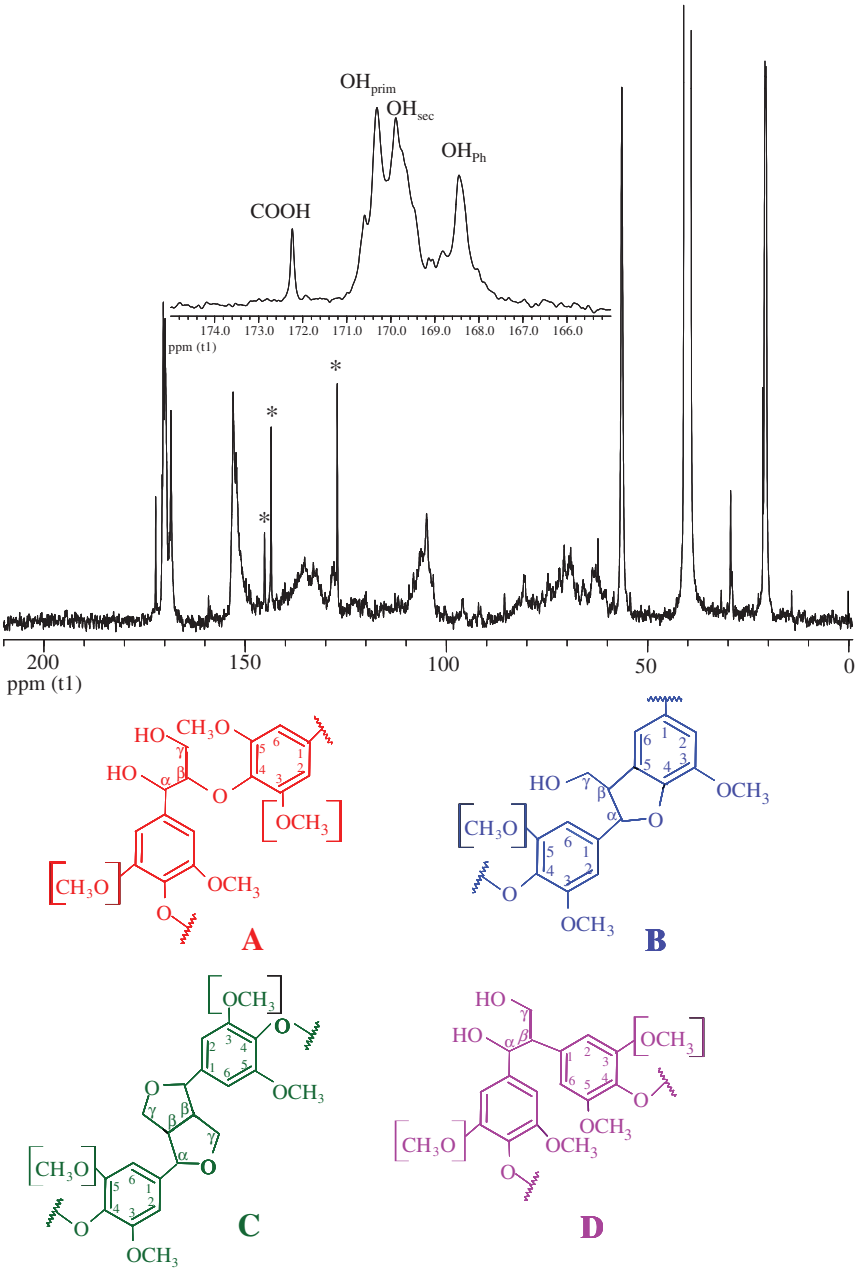


Figure 3 Quantitative ^{13}C NMR spectrum of acetylated L_R (DMSO-d_6, 45°C). The impurities are marked by asterisks and were not considered for integrations. The expanded region at 165–175 ppm shows acetylated phenolic (OH_{ph}) and aliphatic primary (OH_{prim}) and secondary (OH_{sec}) hydroxyl groups. Structures **A**, **B**, **C** and **D** designate β-*O*-4′, β-5′, β-β′ and β-1′ structures, respectively.

acetylated L_R at ca. 5.4–5.5/87–88 ppm and the characteristic cross-peak at ca. 4.65/68.8 ppm assigned to sulphonated BnC in **B′** structures (Figure 2). The sulphonation at the Bn position of a part of S units in L_R is also evidenced by the characteristic down field chemical shift of C2,6 in corresponding structures (Figure 5).

The analysis of quantitative ^{13}C NMR spectra (Figures 2 and 3) also shows a noticeable increase in the relative abundance of S structures in L_R compared to L_{SSL} (Table 3). This observation can be explained, at least partly, by the removal of highly sulphonated monomeric S-type structures in the purification step of L_{SSL} by dialysis (Marques et al. 2009b). However, the proportion of S units in L_R was even higher than that in dioxane lignin (DL) isolated from wood. This is in agreement with data of $KMnO_4$ oxidation of lignins (Table 2) and may be attributed to the topochemical peculiarities of the acidic sulphite process in eucalypt wood. It was highlighted previously that the delignification of vessels, rays and middle lamellae (rich in G units) of hardwood is faster than the secondary

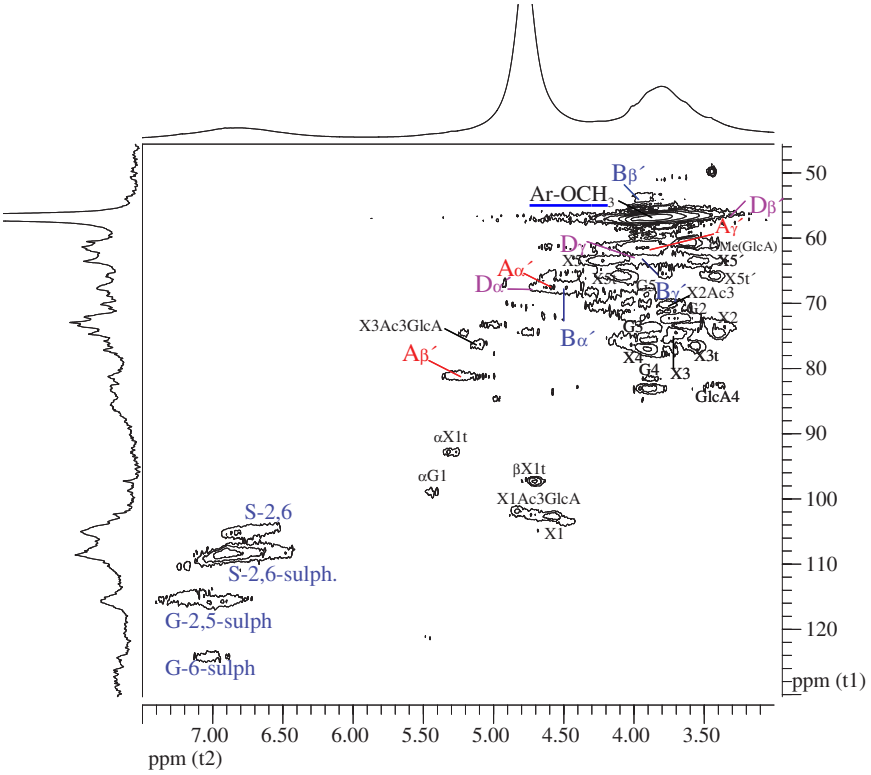


Figure 4 HSQC spectrum of L_{SSL} (D_2O, 50°C). Designations for the main lignin structures are presented in Figures 1 and 2. S-n and G-n designates the signals from syringyl and guaiacyl units, respectively, where n is the number of proton/carbon atom (sulphonated units designated with the index *sulph*). Xn, Gn and GlcAn designates β-D-xylopyranose, β-D-glucopyranose and 4-*O*-methyl-α-D-glucuronopyranose structural units, respectively, where n is the number of proton/carbon atom; the reducing terminal units are designated by letter *t*, and α- and β-isomers are denoted by α and β, respectively (e.g., αX1t means anomeric proton/carbon in α-isomer of reducing β-D-xylopyranose residue). XnAcmGlcA means the proton/carbon *n* in β-D-xylopyranose residue possessing the acetyl group (Ac) at carbon *m* and substituted at *O*-2 by 4-*O*-methyl-α-D-glucuronopyranose residue.

wall of libriforms enriched in S structural units (Glennie 1971; Terashima 1990). Despite sometimes contradicting opinions on the topochemistry of sulphite and sulphate pulping, it is recognised that at the same level of retained lignin, its content in the S2 of sulphite pulp is higher than in kraft pulp (Wood and Goring 1973; Koch et al. 2003). In view of the prevalence of S structures in L_R, it can be concluded that RL in sulphite pulp is localised essentially in the S2. The opposite is true for kraft *E. globulus* pulp, where the RL has lower proportion of S units than wood lignin (Pinto et al. 2003a,b). The relatively high amount of S units in the RL contributes to the easy bleachability of eucalypt sulphite pulp.

The quaternary carbons in aromatic rings as detected by quantitative ^{13}C NMR, which are for structures with alkyl-aryl and aryl-aryl linkages, are in agreement with results of PO (Table 2) as they also indicate more condensed structures in L_R than in DL (Table 3). At the same time, the relative abundance of quaternary aromatic carbons in L_{SSL} is even higher than that in L_R. Accordingly, the degree of lignin condensation itself does not explain the lignin retention in pulp upon cooking. Most probably, the accessibility of lignin in the cell wall towards pulping acid and eventual lignin-carbohydrate (LC) interactions plays an essential role for the retention of RL in pulp fibres.

Glucan and glucuronoxylan are associated with RL according to sugars analysis in L_R (Table 2) and the data of the HSQC spectrum (Figure 5). These are supposed to be chemically linked to RL. The last hypothesis about LC bonds in L_R is confirmed by characteristic cross-signals in the HSQC spectrum at 3.23/61.2 ppm and at 4.56/79.2 ppm assigned to H6/C6 in glucopyranose unit and Hα/Cα in phenylpropane unit, respectively, involved in α-*O*-6 Bn ether linkages (Taneda et al. 1987). The signal of BnC in benzyl ether-type LC structures was shifted to 4.60/81.5 ppm in the HSQC spectrum of non-acetylated L_R (Figure 6), which is in agreement with previously reported assignments in the corresponding structures of lignin-carbohydrate complexes isolated from birch (*Betula pendula*) wood (Balakshin et al. 2011) and eucalypt (*E. globulus*) wood (Miyagawa et al. 2013). Such LC linkages are present

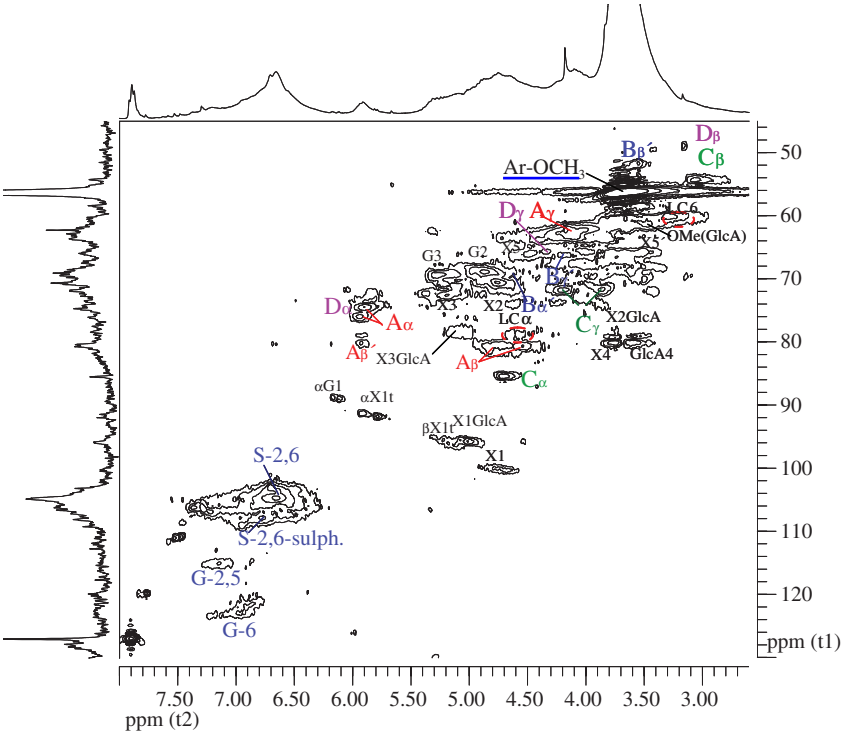


Figure 5 HSQC spectrum of acetylated L_R (DMSO-d_6, 45°C). Designations for the main lignin structures are the same as in Figure 3. Xn, Gn and GlcAn designates acetylated β-D-xylopyranose, β-D-glucopyranose and 4-*O*-methyl-α-D-glucuronopyranose structural units, respectively, where n-number of proton/carbon atom. The reducing terminal units are designated by letter *t*, and α- and β-isomers are denoted by α and β, respectively (e.g., αX1t means anomeric proton/carbon in α-isomer of reducing acetylated β-D-xylopyranose residue). XnGlcA means the proton/carbon *n* in acetylated β-D-xylopyranose residue substituted at *O*-2 by 4-*O*-methyl-α-D-glucuronopyranose residue. LSα and LC6 are cross-signals assigned to benzylic and H6/C6 in glucopyranose, respectively, involved in α-*O*-6 benzyl ether linkage (depicted by the dashed red line).

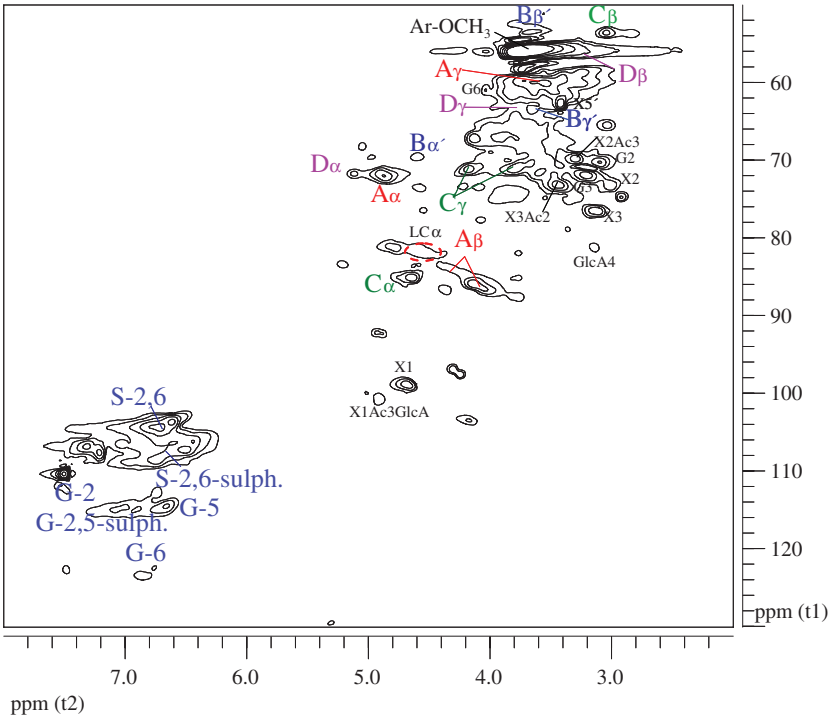


Figure 6 HSQC spectrum of L_R (DMSO-d_6, 45°C). Designations for the main lignin structures are presented in Figure 3. LSα is the cross-signal of benzylic proton/carbon in α-*O*-6 benzyl ether LC linkage (depicted by the dashed red line).

Table 3 Structural analysis of lignins by quantitative ^{13}C NMR (per 100 C_6).

Structural elements	L_{SSL}	L_R	DL[a]
ß-*O*-4 str.	34	45	56
ß-5 str.	6	2	3
ß-ß str.	–	08	13
OCH_3	136	142	147
S:G ratio	72:28	89:11	85:15
ArH	211	213	217
Ar-C	138	135	129
Ar-O	251	252	254
OH_{aliph}	–	117	91
OH_{phen}	–	44	30

[a]Evtuguin et al. (2001).

originally in the cell wall, though their formation during acidic sulphite pulping cannot be completely excluded.

It is noteworthy that two expected cross-peak signals in non-acetylated L_R were not detected, namely Bn proton/Bn carbon involved in ether linkage with xylopyranose (5.10–5.15/81.5–82.5 ppm) and in ester linkage with glucuronopyranose residues (5.5–6.0/75–76 ppm) (Figure 6). This fact, however, does not mean that lignin and glucuronoxylan are not chemically associated. Evtuguin et al. (2003) found that at least a part of eucalypt glucans is linked with xylan by glucosidic (1→2) linkages with terminal 4-*O*-methyl-α-D-glucuronosil residues. The structural association between RL in sulphite pulp and hemicelluloses needs further study.

Conclusions

The residual lignin (L_R sample) isolated from acidic sulphite eucalypt pulp shows structural features, which are closer to dioxan lignin (DL sample) isolated from the same wood rather than to the lignin dissolved in spent sulphite liquor (L_{SSL} sample). In general, L_R contains lower amounts of the main structures (β-*O*-4′, β-5′ and β-β′) than DL and is partially sulphonated. In fact, L_R contains three times less sulphonic groups than L_{SSL}. The main characteristic features of L_R are the relatively high proportion of syringyl (S) structural units, which are strongly condensed at the C6 position, and the noticeable structural association with hemicelluloses via benzyl ether linkages. The features, high amounts of S units in the L_R and high amounts of phenolic structures, contribute probably to the easy bleachability of eucalypt sulphite pulp. Results of this work also call attention again to the difference in chemical structure and allocation of L_R in fibres of sulphite and kraft pulps obtained from eucalyptus wood.

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References

Ämmälahti, E., Brunow, G., Barget, M., Robert, D., Kilpeläeinen, I. (1998) Identification of side-chain structures in a poplar lignin using three-dimensional HMQC-HOHAHA NMR spectroscopy. J. Agric. Food Chem. 46:5113–5117.

Balakshin, M., Capanema, E., Gracz, H., Chang, H.-M., Jameel, H. (2011) Quantification of lignin-carbohydrate linkages with high-resolution NMR spectroscopy. Planta 233:1097–1110.

Berry, R.M., Bolker, H.I. (1987) The topochemistry of acid sulphite pulping. A theoretical approach: Part II. J. Wood Chem. Technol. 7:25–32.

Bock, K., Pedersen, C., Pedersen, H. (1984) Carbon-13 nuclear magnetic resonance data for oligosaccharides. Adv. Carbohydr. Chem. Biotechnol. 42:193–225.

Evtuguin, D.V., Domingues, P.N., Amado, F.L., Pascoal Neto, C., Ferrer Correia, A. (1999) Electrospray ionization mass spectrometry as a tool for lignins molecular weight and structural characterization. Holzforschung 53:525–528.

Evtuguin, D.V., Pascoal Neto, C., Silva, A.M.S., Domingues, P.M., Amado, F.M.L., Robert, D., Faix, O. (2001) Comprehensive study on the chemical structure of dioxane lignin from plantation *Eucalyptus globulus* wood. J. Agric. Food Chem. 49:4252–4261.

Evtuguin, D., Tomás, J., Silva, A., Pascoal Neto, C. (2003) Characterization of an acetylated heteroxylan from *Eucalyptus globulus* Labill. Carbohydr. Res. 338:597–604.

Fletcher, D.E., Johansson, N.G., Basta, J.J., Holm, A.-S., Wackerberg, E. (1997) TCF bleaching of sulfite pulps – new aspects. Tappi J. 80:143–147.

Gellerstedt, G. (1992) Chemical degradation methods: permanganate oxidation. In: Methods in Lignin Chemistry. Eds. Lin, S.Y., Dence, C.W., Springer-Verlag, Berlin. pp. 322–333.

Gierer, J. (1982) The chemistry of delignification. A general concept. Holzforschung 76:43–51.

Glennie, D.W. (1971) Reactions in sulfite pulping. In: Lignins Occurrence, Formation, Structure and Reactions. Eds. Sarkanen, K.V., Ludwig, C.H., Wiley Interscience, New York, pp. 597–637.

Germer, E., Hostachy, J.-C. (2011) State-of-the-art industrial ozone bleaching. Tappsa J. 5:30–37.

Kallmes, O. (1960) Distribution of constituents across the wall of unbleached spruce sulfite fibers. Tappi 43:143–153.

Kilpeläeinen, I., Sipilae, J., Brunow, G., Lundquist, K., Ede, R.M. (1994) Application of two-dimensional NMR spectroscopy to wood lignin structure determination and identification of some

minor structural units of hard- and softwood lignins. J. Agric. Food Chem. 42:2790–2794.

Koch, G., Rose, B., Patt, R., Kordachia, O. (2003) Topochemical investigations on delignification of *Picea abies* L. Karst. during alkaline sulfite (ASA) and bisulphite pulping by scanning UV microspectrophotometry. Holzforschung 57:611–618.

Lisboa, S.A., Evtuguin, D.V., Pascoal Neto, C., Goodfellow, B.J. (2005) Isolation and structural characterization of polysaccharides dissolved in *Eucalyptus globulus* kraft black liquors. Carbohydr. Polym. 60:77–85.

Luthaes, B.F., Myrvold, B.O., Lauten, R.A., Endeshaw, M.M. (2008) ^{1}H and ^{13}C NMR data of benzylsulfonic acids – model compounds for lignosulfonates. Magn. Reson. Chem. 46: 299–305.

Marques, A.P., Evtuguin, D.V., Magina, S., Amado, F.M.L., Prates, A. (2009a) Chemical composition of spent liquor from acidic magnesium-based sulphite pulping of *Eucaplyptus globulus*. J. Wood Chem. Technol. 29:322–336.

Marques, A.P., Evtuguin, D.V., Magina, S., Amado, F., Prates, A. (2009b) Structure of lignosulphonates from acidic magnesium-based sulphite pulping of *Eucaplyptus globulus*. J. Wood Chem. Technol. 29(4):337–357.

Miyagawa, Y., Kamitakahara, H., Takano, T. (2013) Fractionation and characterization of lignin-carbohydrate complexes (LCCs) of *Eucaplyptus globulus* in residues left after MWL isolation: Part II. Analyses of xylan-lignin fraction (X-L). Holzforschung 67:629–642.

Pinto, P.C., Evtuguin, D.V., Pascoal Neto, C., Silvestre, A.J.D. (2002a) Behaviour of *Eucalyptus globulus* lignin during kraft pulping: Part 1. Analysis by chemical degradation techniques. J. Wood Chem. Technol. 22:93–108.

Pinto, P.C., Evtuguin, D.V., Pascoal Neto, C., Silvestre, A.J.D. (2002b) Behaviour of *Eucalyptus globulus* lignin during kraft pulping: Part 2. Analysis by NMR, ESI/MS and GPC techniques. J. Wood Chem. Technol. 22:109–125.

Ralph, S.A., Ralph, J., Landucci, L.L. (2004) NMR database of lignin and cell wall model compounds. Available at http://ars.usda.gov/Services/docs.htm?docid=10491 (accessed 13 April 2009).

Rydholm, S.A. (1965) Pulping Processes. Interscience Publishers, New York.

Sjöström, E. Wood Chemistry: Fundamentals and Applications. Academic Press, New York, 1993.

Stone, J.E. (1955) A study of the lignin removed during a neutral sulphite cook of aspen. Tappi J. 38:610–612.

Süss, H.-U. (2006) Application in TCF sulfite pulp bleaching. In: Handbook of Pulp. Vol. 2. Ed. Sixta, H., Wiley-VCH, Weinheim, pp. 877–880.

Terashima, N. (1990) A new mechanism for formation of a structurally ordered protolignin macromolecule in the cell wall of tree xylem. J. Pulp Paper Sci. 16:J150–J155.

Wood, J.R., Goring, D.A. (1973) The distribution of lignin in fibers produced by kraft and sulphite pulping of spruce wood. Pulp Paper Mag. Can. 74:T309–T313.

Zakis, G.F. Functional Analysis of Lignins and Their Derivatives. Tappi Press, Atlanta, GA, USA, 1994.